Novel genetic causes and pathological mechanisms of neurological and mitochondrial disorders

Summary

Neurological disorders primarily affect and impair the functioning of the brain and/or neurological system. Structural, electrical or metabolic abnormalities in the brain or neurological system can result in a range of clinical symptoms, including brain malformation, neuropathies, encephalopathies, myopathies and muscular dystrophies, movement disorders, and dementias. Neurological disorders affect people of all ages with devastating consequences and leading to major morbidity and mortality. Neurological disorders can have many different causes, including genetic defects, infections, trauma, brain injury, but they can also develop as a result of an unhealthy environment, for example malnutrition. **The main objective of this thesis is to discover and functionally the genetic cause in genetic neurological disorders, in which mitochondria are expected to play a pathological role.** The genetically heterogeneous nature of neurological and mitochondrial diseases makes identifying the disease-causing mutations highly challenging. Mitochondrial diseases are even more complex, as both the mtDNA and the nuclear genome can be involved. We used next-generation sequencing to analyze the mtDNA and the exome in patients with suspected mitochondrial disease as a comprehensive and unbiased approach to identify the genetic cause. Finding the genetic cause is important for diagnosis, prognosis and reproductive options, but understanding the underlying disease mechanisms could reveal novel targets for therapeutic interventions.

Chapter 1 provides an overview of neurological disorders, primarily focusing on those in which defective mitochondria are playing a causative role. These diseases are clinically and genetically heterogeneous, providing considerable limitations to phenotype driven approaches to identify the genetic cause. As mitochondria are under dual genetic control, both the mtDNA and the nuclear genome encode the many hundreds of mitochondrial proteins, each of which can be a candidate in case of mitochondrial diseases. To date, hundreds of nuclear DNA mutations have been identified as cause of mitochondrial diseases and new cases are being resolved on a daily basis, mainly due to the progress in sequencing technology. The impact of next generation sequencing and whole exome or whole genome analysis as effective methods to detect the genetic basis of neurological diseases are being introduced. As these approaches yield huge numbers of variants, accurate filtering strategies and functional models are essential to confirm the role of novel genes and/or variants.

In chapter 2 to 5, I present the results of whole exome sequencing to identify the genetic defects in 4 families with presumably autosomal recessive, neurological and possibly mitochondrial diseases, in which the mtDNA was excluded. Novel genes and/or novel potentially pathogenic variants were further functionally investigated in patient-derived cell lines and in zebrafish models. **Chapter 2** reports on a patient from non-consanguineous Dutch parents, presenting with cerebellar ataxia and atrophy. Exome sequencing revealed that the patient was compound heterozygous for pathogenic variants (c.37G>C; p.D13H and c.946A>T; p.K316*) in the *CWF19L1* gene. Variant p.D13H changed the highly conserved, negatively charged aspartic acid into the positively charged histidine and was predicted to affect protein stability and function. The other

variant led to a premature stop codon p.K316* which resulted in nonsense-mediated mRNA decay. Although the exact function of the C19L1 is still unknown, the nuclear localization in combination with a metallophosphatase domain, which is found in RNA lariat debranching enzymes, suggested a role in mRNA processing. Muscle biopsies showed ragged-red fibers due to accumulation of abnormal mitochondria, which indicated that *CWF19L1* could play a role in mitochondrial structure and function. An additional 27 patients with autosomal recessive cerebellar ataxia (ARCA) were tested for pathogenic variants in the *CWF19L* gene. However, no pathogenic variants were identified in these patients. Our results indicated *CWF19L1* mutations as a novel but rare cause of autosomal recessive cerebellar ataxia. Chapter 3 describes WES in a Moroccan girl of consanguineous parents with optic atrophy and cerebellar atrophy. A novel homozygous mutation was detected in the *SLC25A46* gene (c.283+3G>T), which led to alternative splicing, a frameshift and a premature stop codon. *SLC25A46* mRNA expression showed there is hardly any wild-type transcript present in the patient. Patients fibroblasts showed a fragmented mitochondrial network, confirming a role for *SLC25A46* in mitochondrial dynamics. An additional 10 patients with optic atrophy and cerebellar atrophy, who did not carry mtDNA and *OPA1*mutations, were tested for pathogenic mutations in the *SLC25A46* gene. However, no additional pathogenic variants were identified. Our findings confirmed *SLC25A46* as a novel mitochondrial cause for optic atrophy spectrum disorder. In Chapter 4 a patient from non-consanguineous Dutch parents is described, presenting with slowly progressive neurodegeneration, cerebellar ataxia, chorea dystonic movements, polyneuropathy, type 1 diabetes and OXPHOS complex IV deficiency in muscle. Compound heterozygous splice site mutations were identified by WES in the *COX18* gene (c.720 G>A and c.828+3 G>C). COX18 catalyzes the proper translocation of the C-terminus of COX2 across the mitochondrial inner membrane, which makes essential for proper COX assembly. Both mutations led to abnormal splicing and a premature stop codon, explaining the severe complex IV deficiency observed in muscle of the patient. Our results indicated *COX18* mutations as a novel cause of isolated COX deficiency. Chapter 5 reports on a Turkish patient from a consanguineous family with a severe neurodegenerative condition, including cerebellar atrophy, spasticity, feeding problems and epilepsy. WES combined with homozygosity mapping identified possible pathogenic mutations in 2 candidate genes, *HKDC1* and *MED20*. *MED20* mutations have been associated in one case of infantile basal ganglia degeneration and brain atrophy. Our patient presented with more severe neurological symptoms. Therefore, the role of *HKDC1* in the phenotype was investigated by performing morpholino (MO) mediated knockdown experiments in zebrafish. Treated zebrafish displayed microcephaly and small eyes, which nicely resembled the human microcephaly phenotype. Our data indicated that defects in *HKDC1* as well as *MED20* could explain the severe cerebellar atrophy, neuronal degenerative condition, spasticity, feeding problems and epilepsy observed in our patient. It is unclear so far which of them defines the disease manifestation or that it is combined effect of the two.

In Chapter 6 we described a summary of all gene and gene defects identified in a cohort of 119 patients, mostly children, with either a high suspicion of a mitochondrial disorder or with a disease phenotype in which mitochondrial defects are part of the differential diagnosis. In this cohort, a next generation sequencing based approach was used in two steps. Firstly, the mtDNA was screened for mutations, and secondly, if negative for pathogenic mtDNA mutations, Whole Exome Sequencing (WES) was performed. mtDNA point-mutations and INDELs were detected in 20% of the patient-cohort, and a disease-causing nuclear gene mutation in 50% of the patients, implying an overall diagnostic yield of 70%. In an additional 7% of the WES-cohort, a variant with a possible lead to the patient's phenotype was found and further laboratory validation could confirm a role in disease. As 31% of the disease-causing genes were at the date of genetic diagnosis not present in the MitoCarta database and in some cases more than 1 genetic cause was present, this disqualifies gene panel based approaches and is a plead for a complete analysis of the entire exome, providing a comprehensive overview of all relevant variants. Our results demonstrate that WES is the preferred approach to establish a genetic diagnosis in genetically heterogeneous neurological and/or mitochondrial disease.

The general discussion (Chapter 7) discusses first the importance of next generation sequencing, WES and WGS to identify the genetic cause in neurological diseases with a potential involvement of mitochondria. These developments have resulted in a paradigm shift in the diagnostics of genetically heterogenous disorders, which was phenotype-based and is now largely genotype-based. Impressive progress has been made towards understanding the basis of many neurological diseases. Particular advantages and limitations in NGS are highlighted and the clinical and research applications of these sequencing platforms for neurological disorders are discussed. The advances in finding genetic variants emphasized the need for model systems that give experimental evidence for the role of these variants in disease and characterize the underlying pathophysiological mechanisms. The second part of chapter 7 discusses a variety of model systems, *in vitro*, *in vivo* and *in silico*, which are utilized in neurological disorders. The advantages and limitations of each of these models in studying human neurological diseases based on the extent to which aspects of human phenotypes are recapitulated, are being discussed.